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ORIGINAL ARTICLE

Pregnancy-associated plasma protein A gene polymorphism in pregnant women with preeclampsia and intrauterine growth restriction



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Received 17 December 2014; accepted 13 August 2015

Available online 28 September 2015

KEYWORDS

Gene;
Intrauterine growth
restriction;
PAPP-A;
Polymorphism;
Preeclampsia

Abstract Preeclampsia (PE) and intrauterine growth restriction (IUGR) are still among the most commonly researched titles in perinatology. To shed light on their etiology, new prevention and treatment strategies are the major targets of studies. In this study, we aimed to investigate the relation between gene polymorphism of one of the products of trophoblasts, pregnancy-associated plasma protein A (PAPP-A) and PE/IUGR. A total of 147 women (IUGR, $n = 61$; PE, $n = 47$; IUGR + PE, $n = 37$; eclampsia, $n = 2$) were compared with 103 controls with respect to the sequencing of exon 14 of the PAPP-A gene to detect (rs7020782) polymorphism. Genotypes "AA" and "CC" were given in the event of A or C allele homozygosity and "AC" in A and C allele heterozygosity. Our findings revealed that the rate of AA, CC homozygotes, and AC heterozygotes did not differ between groups. Moreover, there was no difference in the distribution of PAPP-A genotypes among the patients with IUGR, PE, IUGR + PE, or eclampsia. Finally, birth weight, rate of the presence of proteinuria, and total protein excretion on 24-hour urine were similar in the subgroups of AA, AC, and CC genotypes in the study group. Our study demonstrated no association between PAPP-A gene rs7020782 polymorphism and PE/IUGR. Copyright © 2015, Kaohsiung Medical University. Published by Elsevier Taiwan LLC. All rights reserved.

Conflicts of interest: All authors declare no conflicts of interest.

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<http://dx.doi.org/10.1016/j.kjms.2015.08.005>

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Introduction

Although several associated theories have been proposed about the underlying mechanisms of preeclampsia (PE), a disease generally defined as pregnancy-induced hypertension with albuminuria arising after the 20th week of gestation, its etiology has yet to be definitively defined [1]. Today, PE is thought to result from a composition of immunologic, inflammatory, nutritional, and genetic factors that lead to the failure of the action of migratory interstitial and endovascular trophoblast into the walls of the spiral arterioles [2–4].

Intrauterine growth restriction (IUGR), affecting ~5–10% of pregnancies, is usually defined as the situation when the fetus is below the 10th percentile on the curve of weight for gestational age [5]. Maternal, fetal, placental, and external factors combined with the genetically predetermined growth potential organize the normal fetal growth, and failures of these factors result in IUGR and associated disorders [6].

The insulin-like growth factors (IGFs) play a considerable role in the growth of fetoplacental tissues throughout gestation [7]. Six insulin-like growth factor binding proteins (IGFBPs) organize IGF activities by escalating the half-life, localization, and migration of IGFs in particular tissues [7,8]. Pregnancy-associated plasma protein-A (PAPP-A), a zinc-binding metalloproteinase, releases IGF1 by cleaving the IGFBP-4/IGF 1 complex in order to modulate local IGF1 bioavailability [9]. Moreover, the potential of the differential regulation of decidual PAPP-A in arranging IGF 2 bioavailability at the trophoblast–decidual interface reveals that maternal PAPP-A in the endometrial stroma and the trophoblastic tissues may have an influence on placental and fetal growth and development during implantation and pregnancy [10,11].

Proper interference between maternal endometrium, and trophoblastic and embryonic tissues is mandatory to ensure successful pregnancy outcomes. The failures in the processing of proteins such as PAPP-A, which may result in defective placentation, may pose unfavorable situations such as PE/IUGR. We performed this study to evaluate the potential association between the gene polymorphism (rs7020782) of PAPP-A and PE/IUGR.

Methods

This prospective study was conducted on 250 women who presented for prenatal care between January 2010 and December 2013 to the Gynecology and Obstetrics Department of Ege University, Izmir, Turkey. The protocol of the study was approved by the Ethics Committee for Human Studies at Ege University, and informed consent was obtained from all participants. The inclusion criterion was a diagnosis of PE, IUGR, PE with IUGR, or eclampsia. Women with multiple pregnancies and pregnancies with congenital and chromosomal abnormalities were excluded from the study. The control group consisted of patients with normal blood pressure and an appropriate weight for gestational age fetus.

PE was defined as a persistent systolic blood pressure of 140 mmHg and a diastolic blood pressure of 90 mmHg or

higher, taken at least 6 hours apart, after 20 weeks of gestation in a previously normotensive patient. Proteinuria was defined as urine protein concentration of ≥ 300 mg/dL or 1+ on a urine dipstick in at least two random specimens collected at least 4 hours apart [12]. Total protein excretion on 24-hour urine was also calculated. IUGR was defined as estimated fetal weight below the 10th percentile on ultrasound together with birth weight below the 10th percentile of the standard growth curve [5]. Finally, eclampsia was defined as the development of convulsions and/or unexplained coma during pregnancy or postpartum in patients with signs and symptoms of PE [13].

Genomic deoxyribonucleic acid (DNA) was isolated from peripheral blood cells using standard techniques. Forward 5'-GCACTTCGCATGTGAGAAAA-3' and reverse 5'-GAGCTCAGCACCTGCACATA-3' were used in order to amplify the region surrounding rs7020782 polymorphism in the PAPP-A gene [10,14]. Polymerase chain reaction (PCR) was performed in a 25- μ L reaction mix containing 5 μ L of extracted DNA, 2.5 μ L buffer (10 \times Taq Buffer with KCl), 2 μ L MgCl₂ (25mM), 1 μ L dNTP (10mM), and 1.25 μ L (10 pmol/ μ L) forward and reverse primer 0.5 μ L (5 U/ μ L) Taq-Polymerase, and 11.5 μ L distilled water. PCR conditions were as follows: 95°C for 11 minutes, then 35 cycles of 95°C for 30 seconds, 57°C for 30 seconds, 72°C for 30 seconds, followed by extension at 72°C for 5 minutes. PCR products were visualized on a 2% agarose gel by electrophoresis and purified before cycle sequencing. All PCR products were sequenced with the dye termination method using a DNA sequencing kit (Perkin-Elmer, Foster City, CA, USA) and analyzed using the ABI Prism 3100 sequence analyzer (Applied Biosystems, Foster City, CA, USA). Genotypes "AA" and "CC" labels were given in the event of A or C allele homozygosity and "AC" in the event of A and C allele heterozygosity.

Statistical analysis was performed with the SPSS version 11.0 statistical software package (SPSS Inc., Chicago, IL, USA). Kolmogorov–Smirnov test was used to detect normal distribution. Continuous parameters were expressed as mean \pm standard deviation when normally distributed. Categorical results were expressed as percentages. For comparisons, Chi-square, analysis of variance, Kruskal–Wallis, and Mann–Whitney *U* tests were used, where appropriate.

Results

A total of 147 women (61 IUGR, 47 PE, 37 IUGR + PE, 2 eclampsia) were compared with 103 controls. Patients in the study group delivered at earlier gestational ages and had infants with significantly lower birth weights (Table 1). The rate of AA, CC homozygotes, and AC heterozygotes did not differ between study and control groups ($p = 0.966$, Chi-square test; Table 2).

Furthermore, there was no difference in the distribution of PAPP-A genotypes among the patients with IUGR, PE, IUGR + PE, or eclampsia ($p = 0.942$, Chi-square test; Table 3). Additionally, birth weight, rate of the presence of proteinuria, and total protein excretion on 24-hour urine were similar in the subgroups AA, AC, and CC genotypes in the study group (Table 4).

Table 1 Characteristics of groups.

	Study group (<i>n</i> = 147)	Control group (<i>n</i> = 103)	<i>p</i>
Age (y)	29.8 ± 6.0	28.6 ± 5.5	NS
Gravida	2.1 ± 0.9	1.9 ± 0.9	NS
Parity	0.7 ± 0.6	0.9 ± 0.6	NS
Body mass index (kg/m ²)	25.0 ± 5.0	24.8 ± 3.8	NS
Gestational age at delivery (wk)	34.9 ± 3.2	38.1 ± 2.6	< 0.001*
Birth weight (g)	1895 ± 650	3163 ± 647	< 0.001*

Data are presented as mean ± SD.

* Statistically significant (Mann–Whitney *U* test).

NS = not significant; SD = standard deviation.

Table 2 Distribution of PAPP-A genotypes in the study and control groups.

	AA	AC	CC	Total
Study group	78 (53.1)	59 (40.1)	10 (6.8)	149 (100)
Control group	53 (51.5)	43 (41.7)	7 (6.8)	103 (100)

Data are presented as *n* (%).

Table 3 Distribution of PAPP-A genotypes among various groups.

	AA	AC	CC	Total
IUGR	35 (57.4)	21 (34.4)	5 (8.2)	61 (100)
PE	24 (51.1)	20 (42.6)	3 (6.4)	47 (100)
IUGR + PE	18 (48.6)	17 (45.9)	2 (5.4)	37 (100)
Eclampsia	1 (50)	1 (50)	0 (0)	2 (100)

Data are presented as *n* (%).

IUGR = intrauterine growth restriction; PE = preeclampsia.

Discussion

The etiologies of PE and IUGR are multifactorial, with potential variations in genes involved in placental development, blood pressure regulation, or endothelial functioning. The findings of recent investigations revealed that some of the maternal polymorphisms including

C-reactive protein gene, angiotensinogen gene, tumor necrosis factor receptor gene, and vascular endothelial growth factor family genes were related to higher risks of PE occurrence [15–18]. However, this relation was not distinct when examined in some variants of multidrug resistance 1, interleukin-10 (G-1082A) promoter, and endothelial nitric oxide synthase genes [19–21]. Like PE, the association of some maternal gene polymorphisms and IUGR were revealed in contemporary trials [22,23]. In a well-designed study, maternal deletion allele of angiotensin-converting enzyme gene was found to be associated with fetal growth restriction [24]. Although not confirmed in our study, these results indicate the prospect that genetic factors may have important roles in PE and IUGR pathogenesis.

The PAPP-A gene is located in chromosome 9q33.1 and contains 22 exons [25]. The clinical importance of the polymorphism of this gene was primarily searched in cardiology studies and determined as an independent risk factor for acute myocardial infarction [26]. Later, studies in perinatal medicine analyzing other exons were conducted. In one of the trials that aimed to evaluate PAPP-A polymorphisms in patients with risk pregnancies (preterm labor and PE), exon 5 and exon 7 were examined, but no divergence from the wild-types were detected [27]. In another study, exon 2 and exon 6 were assessed, and significantly higher frequency of Cys327Cys (rs2375498) polymorphism in exon 2 were found in patients with PE [28].

To the best of our knowledge, our study represents the primary data about the association of rs7020782 polymorphism in exon 14 of PAPP-A gene and IUGR/PE. This variation was initially determined by Frosk et al [29], and its relationship with ischemic cerebrovascular disease was proved [30]. Additionally, some association—although not at a significant level—was determined in patients with recurrent pregnancy loss [10]. According to our final data, tyrosine/serine mutation (rs7020782 polymorphism in exon 14) does not seem to be a part of either PE or IUGR.

The effect of maternal serum PAPP-A levels on pregnancy outcomes was broadly studied. In a recently published review, decreased serum levels in all trimesters were found to be a risk factor for both PE and IUGR, whereas some authors reported elevated third trimester levels in preeclamptic pregnancies [31,32]. Although appearing as one of the limitations of our study, these conflicting results and the reported uncorrelation between PAPP-A polymorphism and serum PAPP-A levels prompted us to forego performing a maternal serum PAPP-A analysis [10]. The

Table 4 Features of AA, AC, and CC genotypes in the study group.

	AA genotype	AC genotype	CC genotype	<i>p</i>
Birth weight (g)	1970 ± 706	1769 ± 590	2067 ± 455	0.161 ^a
Presence of proteinuria	55.1	62.1	60	0.714 ^b
24-h urine protein (g/d)	3.06 ± 3.07	5.71 ± 5.82	6.15 ± 2.60	0.107 ^c

Data are presented as mean ± SD or (%).

ANOVA = analysis of variance; SD = standard deviation.

^a Indicates ANOVA.

^b Indicates Chi-square test.

^c Indicates Kruskal–Wallis test.

limited sample size, particularly in the CC genotype group, is another limitation. Multicentral studies may put forward solutions, particularly with regard to the consequences of the low frequency of this type of genetic variants.

PAPP-A is a product of growing trophoblasts and indirectly induces aggression to the endometrium [33]. Although they have multifactorial underlying etiologies, PE and IUGR are partly thought to be disorders of abnormal early trophoblastic invasion. In this prospective study, we included pregnant women with PE, IUGR, IUGR with accompanying PE, or eclampsia, and evaluated the potential association of these disorders and PAPP-A gene (rs7020782) polymorphism. However, our results showed no relation. Future studies evaluating other gene polymorphisms may reveal more valuable data about PE and IUGR.

Acknowledgments

This work was supported by the Research Foundation of Ege University.

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